important implications for muscular dystrophy and its therapy. Our previous TPA studies, detecting the microsecond dynamics of phosphorescent-labeled actin, showed that both proteins have novel effects on actin flexibility, with utrophin more effective than dystrophin (Prochniewicz et al., 2009, *PNAS*). We have now compared the effects of the isolated actin-binding domains of dystrophin, ABD1 and ABD2. TPA shows that the enhanced rate of actin rotational dynamics is induced primarily by ABD1, while both ABD1 and ABD2 contribute to the restriction in rotational amplitude. Disease-causing point mutations in ABD1 decrease the effects on actin's rotational rate. We propose that this in turn causes the dystrophin-actin complex to be less resilient and thus less able to prevent damage to the muscle cytoskeleton during contraction. Finally, we have attached probes directly to ABD1 in dystrophin and utrophin, to detect changes in structure upon actin binding. High-resolution distance measurements, provided by DEER, show that the two lobes (calponin-homology domains) within ABD1 undergo a dramatic opening upon actin binding, helping to resolve a previous controversy (Lin et al., 2011, PNAS). Analogous studies with dystrophin show more subtle changes upon actin binding, providing insight into the structural and functional differences in dystrophin and utrophin.

1899-Pos Board B669

Caldesmon Stabilizes Nascent Actin Filaments and Promotes Branching by Arp2/3 Complex

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Caldesmon is an actin-binding protein found in nearly all vertebrate cells. The heavy caldesmon isoform, which is specific to smooth muscle cells, is known to inhibit actomyosin interactions in vitro in a phosphorylation-dependent manner. However, possible roles of caldesmon as a regulator of actin mechanics and turnover are being explored, and the effects of caldesmon on actin dynamics and structure are not well understood.

We have recently demonstrated that polymerizing actin undergoes an irreversible structural transition (termed ''maturation''), and that the caldesmon C-terminal fragment, H32K, if added during the early stage of actin polymerization, prevents this maturation process (Huang et al., 2010 J Biol Chem 285(1):71-79). Actin filaments stabilized in this ''nascent'' state by H32K appear rough under electron microscopy and exhibits attenuated pyrene fluorescence enhancement compared to normal, mature F-actin, but H32K does not otherwise affect the polymerization kinetics of actin (Collins et al., 2011 Bioarchitechture 1(3):127-133).

Both phosphorylated caldesmon and the actin-branching protein complex Arp2/3 are present at the leading edge of motile cells, where actin assembly provides the force needed to protrude the plasma membrane. Here, we study the interaction of H32K-stabilized nascent F-actin with Arp2/3 complex. By direct visualization of actin assembly in the presence of Arp2/3 complex using confocal fluorescence microscopy, we show that actin polymerized in the presence of H32K exhibits increased branching activity. We propose that the observed change is a result of the structural state of the young actin filament, which is stabilized by the early added H32K. This effect suggests a role of caldesmon as a regulator of actin structure, in turn dictating the interaction of other actin-binding proteins with actin.

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Structural and Biochemical Study of the Interaction Between Actin and the Mammalian Formin FMNL3

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Formins are a class of proteins that influence the rate of actin filament nucleation and elongation. Mammals possess 15 formin isoforms, providing a myriad of possibilities for regulating actin-based structures in cells. The dimeric formin homology 2 (FH2) domain is capable of altering the nucleation rate of new actin filaments and subsequently influences filament elongation via direct interaction with the barbed end of an actin filament. The FH2 domain moves processively with the barbed end as the filament elongates. Our goal in this study is to examine FMNL3's interaction with actin to uncover mechanistic details of formin regulated actin elongation. The FH2 domain of FMNL3 that forms a stable interaction with tetramethylrhodamine-malamide labeled actin (TMR-actin) and has been crystallized and the 3.3 Å resolution structure has been solved. The crystal structure indicates that FMNL3 dimerizes to form a ring around the barbed end of two actin subunits. The formin/actin interface is comprised of multiple interactions that are found in three distinct regions of the FH2 domain. Mutational analysis identified key residues in FMNL3/actin binding as well as those essential for elongation activity. The study of these residues has led us to propose a more detailed model for the interaction of the FH2 domain with actin. A previous study with Bni1p, revealed an FH2 dimer conformation spanning three actin subunits. This structure supports the model of a formin tracking with the growing end of an actin filament by taking steps with the addition of each monomer. The FMNL3/actin complex represents an intermediate step in the formin stepping mechanism in which two actin subunits are aligned side-by-side surrounded by a symmetric FH2 dimer. This structure reveals mechanistic details of FH2 mediated actin filament elongation via processive capping.

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Tropomyosin and Caldesmon Enhance the Binding Force of Unphosphorylated Myosin to Thin Filaments

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Smooth muscle is unique in its ability to maintain force for long periods of time at low energy consumption. This property, called the latch-state, has been postulated to be a consequence of the dephosphorylation of myosin molecules when attached to the actin thin filament. Alternatively, unphosphorylated (unPHOS) myosin could potentially attach to the thin filaments, contributing to force maintenance. Thus, in this study we verified if unPHOS myosin can bind to actin in presence of tropomyosin and caldesmon and we measured its binding force using the laser trap. Briefly, a microsphere captured in a laser trap was attached to an actin filament. The filament was brought in contact with a pedestal coated with unPHOS myosin. The pedestal was then moved away from the trap at constant velocity. The microsphere followed the pedestal until the force exerted by the trap on the microsphere was sufficient to overcome the binding force of myosin to the actin filament. At this point, the microsphere sprang back into the trap center. The force of unbinding (Funb) was calculated as the product of the trap stiffness and the maximal distance between the trapped microsphere and the trap center. Funb was normalized by the number of myosin molecules estimated per actin filament length. Funb of unregulated actin filaments (0.118 ± 0.007 pN; mean $\pm$ SE) was enhanced in presence of tropomyosin (0.16 ± 0.008 pN; $p<0.05$), caldesmon (0.169 ± 0.017 pN; $p<0.05$) and a mix of both regulatory proteins (0.174 ± 0.022 pN; $p<0.05$). Our data demonstrate that unPHOS myosin binds to regulated actin filaments and that each regulatory protein increases this binding force but that their effect is not cumulative. Thus, the actin regulatory proteins might play a major role in the latch-state, by enhancing the binding of unPHOS myosin.

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Novel Modulation of the S.Cerevsiae Actin-Cyctokeleton by Heterologous Expression of the Short N.Crassa Tropomyosin

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Tropomyosins are actin-regulatory proteins found in eukaryotes from yeast to man. They are best understood in their role in the regulation of muscle contraction in higher eukaryotes. However, only a small subset of the 20 or more isoforms of tropomyosin found in man are involved in muscle regulation. Their extensive diversity indicates their importance in regulating the actin cytoskeleton, a role in which their function is only beginning to be understood.

Saccharomyces cerrevisiae provides a simple model system for trying to understand the functioning of tropomyosins in regulating the actin cytoskeleton. It has two tropomyosins, the larger 5 actin spanning TPM1 and smaller 4 actin spanning TPM2. Knockout of the major TPM1 isoform produces defects in cell division and hence cell size, whilst knockout of the minor TPM2 isoform has no morphological effect. A double knockout is lethal. Previous studies of heterologous expression of other tropomyosins in a ΔTPM1 background have all produced unremarkable 'rescue' of the knockout effects.

We have previously reported that *Neurospora crassa* also has two tropomyosins, but in this case the larger is a 4 actin spanning and the shorter an unusual 3 actin spanning tropomyosin. Heterologous expression of this short *N. crassa* tropomyosin in ΔTPM1 cells does not produce rescue. Instead we observe